

Appl. No. 09/981,636
Amdt. Dated September 27, 2005
Reply to Office Action of June 15, 2005

Amendments to the Claims:

Please amend claims 1, 75, 92, and 93.

This listing of claims will replace all prior versions, and listings, of claims in the application.

These amendments introduce no new matter and support for the amendment is replete throughout the specification and claims as originally filed. These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter, or agreement with any objection or rejection of record.

Listing of Claims:

1. (Currently amended) A method of detecting ligand internalization into a cell, said method comprising:
 - i) contacting said cell with a ligand and a reporter, wherein the reporter non-covalently couples to the ligand;
 - ii) dissociating the reporter from non-internalized ligand bound to a surface of said cell and removing dissociated reporter from the surface of said cell while said non-internalized ligand remains bound to the cell surface; and
 - iii) detecting the presence of the reporter remaining in said cell, whereby the presence of the reporter indicates that said ligand is internalized into said cell.
2. (Previously presented) The method of claim 1, wherein contacting the cell with the ligand and the reporter comprises:
 - contacting said cell with a ligand comprising an epitope tag; and
 - contacting the ligand with a reporter comprising a moiety that binds said epitope tag.
3. (Original) The method of claim 1, wherein said ligand is a ligand that binds to a cell surface receptor.
4. (Withdrawn) The method of claim 1, wherein said ligand is a peptide.
5. (Previously presented) The method of claim 1, wherein said ligand is selected from the group consisting of an antibody, scFv, Fv, Fab, a monoclonal antibody, a cytokine, and a growth factor.

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6. (Previously presented) The method of claim 1, wherein said ligand is a member of a combinatorial library.
7. (Previously presented) The method of claim 6, wherein said combinatorial library comprises a combinatorial chemical library, a recombinant library, or a phage display library.
8. (Previously presented) The method of claim 1, wherein said reporter is non-covalently coupled to the ligand via an epitope tag.
9. (Previously presented) The method of claim 8, wherein the epitope tag is selected from the group consisting of a His-tag, a Flag-tag, an HA-tag, a myc-tag, and a DYKDDDDK epitope.
10. (Previously presented) The method of claim 1, wherein the reporter is selected from the group consisting of an enzyme, a colorimetric label, a fluorescent label, a luminescent label, a radioactive label, a nanoparticle, a spin label, a magnetic bead, and a liposome.
11. (Previously presented) The method of claim 8, wherein said epitope tag is a hexahistidine (His-6) tag and said reporter is a liposome comprising a nitrilotriacetic acid (NTA) lipid or an iminodiacetic acid (IDA) lipid.
12. (Previously presented) The method of claim 8, wherein said ligand is an antibody and said epitope tag is attached to said antibody through a covalent linkage to protein A or protein G.
13. (Original) The method of claim 1, wherein said cell is a cancer cell.
14. (Previously presented) The method of claim 1, further comprising:
 - iv) identifying the ligand that is internalized into said cell.
15. (Previously presented) The method of claim 14, wherein identifying the ligand comprises determining the amino acid sequence of the internalized ligand or determining the sequence of a nucleic acid encoding said ligand.
- 16-72 (Cancelled)

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73. (Withdrawn) The method of claim 8, wherein the epitope tag comprises a polyhistidine tag, and wherein the noncovalent bond comprises a metal chelation bond between the reporter and the polyhistidine tag.
74. (Withdrawn) The method of claim 73, wherein the reporter is selected from the group consisting of an enzyme, a colorimetric label, a fluorescent label, a luminescent label, a radioactive label, a nanoparticle, a spin label, a magnetic bead, and a liposome.
75. (Currently amended) The method of ~~claim 1~~ claim 73, wherein the reporter comprises a metal ion complexed to a chelator selected from the group consisting of NTA, IDA, a C-substituted derivative of NTA, and a C-substituted derivative of IDA.
76. (Withdrawn) The method of claim 75, wherein the metal ion comprises a divalent ion of Cu, Ni, Co or Zn.
77. (Previously presented) The method of claim 2, wherein the ligand and the reporter are combined to form a non-covalent bond prior to the contacting step.
78. (Previously presented) The method of claim 2, wherein the ligand and the reporter are combined to form a non-covalent bond during the contacting step.
79. (Previously presented) The method of claim 1, wherein the method is performed in a microtiter plate.
80. (Withdrawn) The method of claim 1, wherein detecting the presence of the reporter comprises performing scintillography or autoradiography.
81. (Previously presented) The method of claim 1, wherein detecting the presence of the reporter comprises performing fluorimetry, flow cytometry or fluorescent microscopy.
82. (Withdrawn) The method of claim 1, wherein detecting the presence of the reporter comprises determining cell proliferation or cell mortality.
83. (Previously presented) The method of claim 1, wherein detecting the presence of the reporter comprises isolating the cell comprising the internalized ligand.
84. (Previously presented) The method of claim 1, wherein detecting the presence of the reporter comprises a quantitative determination.

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85. (Previously presented) The method of claim 1, wherein detecting the presence of the reporter comprises:

a) performing a first detecting step prior to dissociating reporter from the non-internalized ligand; and,

b) performing a second detecting step after dissociating reporter from the non-internalized ligand.

86. (Previously presented) The method of claim 1, wherein contacting the cell with the ligand and the reporter comprises contacting the cell with at least two different ligands.

87. (Previously presented) The method of claim 1, wherein steps i, ii and iii are performed on a plurality of cells.

88. (Previously presented) The method of claim 87, wherein detecting the presence of the reporter comprises detecting one or more cells comprising the reporter.

89. (Previously presented) The method of claim 88, wherein the reporter comprises a fluorescent label, and wherein the one or more cells are detected by flow cytometry, fluorescent microscopy, or fluorescence-activated cell sorting.

90. (Withdrawn) The method of claim 88, wherein the reporter comprises a magnetic bead, and wherein the one or more cells are detected by magnetometry or by magnetic separation.

91. (Withdrawn) The method of claim 88, wherein the reporter comprises a radioactive label, and wherein the one or more cells are detected by autoradiography.

92. (Currently amended) The method of claim 87, further comprising isolating from the plurality of cells, a member-cell that internalized the ligand.

93. (Currently amended) The method of claim 87, wherein detecting the presence of the reporter comprises quantification of the reporter present in ~~said cell~~ a member cell of the plurality of cells.